THE DEVELOPMENT OF WINGS OF CERTAIN BEETLES, AND SOME STUDIES OF THE ORIGIN OF THE WINGS OF INSECTS.

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(*Continued from Vol. XII, page 243.*)

The downward growth of the wing continues until early in the prepupal period, when it reaches the base of the leg bud, which prevents further growth in this direction (Figs. 8, 21). The wing now exists as a double-walled pad lying between the hypoderm and cuticle, extending downward and backward, as far as the base of the leg and with the basement membranes of the two layers usually closely pressed together, except at the places where tracheæ have entered the wing, forming vein cavities. The nuclei of the two walls still lie at several different planes and the outer (upper) wall is somewhat the thicker, while the bases of the cells are abruptly narrowed and thread-like.

The later stages in the development of the wings of *T. plastographus* and of *D. valens* are very much as have been described by various authors for the Coleoptera and Lepidoptera. With the beginning of the prepupal period the growth of the wing becomes accelerated. With the lengthening of the wing the nuclei begin to be drawn up into one row near the outer ends of the cells, while the cells themselves become very much elongated and drawn out in places (Figs. 9, 10). Shortly before pupation this process becomes completed and the cells have the characteristic "fringed" appearance of the pupal hypodermis described by Verson (1904). The wing is now greatly wrinkled and folded, the vein cavities are greatly enlarged and filled with hæmolymph and leucocytes, both of which also circulate in the spaces between the elongated cells. The basement membrane, which throughout the development of the wing is very thin and not easily discernable, becomes more or less degenerated during the prepupal period and in places the bases of the cells either end free (Fig. 19), or become fused and anastomosed with each other (Figs. 11, 22).

Early in the last stage there is secreted all around the body, underneath the dense outer chitin, an inner layer of soft spongy or stringy chitin which takes a faint stain with hæmatoxylin. This chitin is especially pronounced around the wing buds, which it entirely surrounds, at first more or less completely filling the cavity between the wing and the hypodermis.

(*d*) *Formation of the Veins.* — At about the beginning of the prepupal period, or shortly before, the vein cavities begin to be formed. Several branches arise from the trachea at the base of the wing and push into the wing pad, the two layers of the basement membrane separating as the trachea enters (Fig. 38). At the same time that the tracheæ enter the wing the tracheoles uncoil and accompany the tracheæ. This manner of the formation of the veins, which I have observed in *T. plastographus* and *D. valens* and also in *Bruchus* sp. and the Pepper beetle, differs from the account of Tower, who found that the cavities were formed before the tracheæ develop and enter the wing, whereas, so far as I have been able to observe, the development of the two is coincident.

3. THE TRACHEAL SYSTEM.

According to Comstock and Needham, working on *Hippodamia 13-punctata*, there are in the wings of Coleoptera, as has been so often shown to be the case in the Lepidoptera, two systems of tracheation, a temporary system of tracheoles, and the permanent wing tracheæ, which develop after entering the wing a system of tracheoles of their own; the temporary system of tracheoles being much less highly developed than in the Lepidoptera. The permanent tracheæ enter the wing during the last larval stage, when the wing is well developed and at the beginning of its rapid extension.

While they found the two distinct systems in the Coleoptera, they found that in wings developing externally like those of a dragonfly, "the principal tracheæ pass out very early into the wing bud, branching freely and forming by multitudinous terminal anastomoses a network of capillary tracheoles," no temporary tracheoles being found. They believe that the development of the latter is due to and depends on the confinement of the wing within narrow quarters inside the body and to its small size. Their observations on the Coleoptera were corroborated by the researches of Tower. I have found evidence leading me to the conclusion that there is no fundamental difference between the development of the tracheoles and that of the tracheæ, but that the two systems merge into one another. I will reserve this, however, for a later communication.

(*a*) *The Tracheoles.* — In all the larvæ that I have examined there is from the very beginning a close connection between the wings and the tracheal system. Comstock and Needham found (in *Hippodamia 13-punctata*) that the wing fundament had at first "no connection with or approximation to any trachea," this connection not occurring until the wing disc had invaginated well into the body, approaching a lateral tracheal trunk, and evagination had begun. Needham found that in *Mononychus vulpeculus* the wing bud differed from the leg bud only in having no tracheæ at or near its inner surface. This I think is an unusual condition and not to be found in many Coleoptera. In all the Coleopterous larvæ examined by Tower (1903), even in the earliest stages the wing fundament received a distinct branch from the tracheal trunk; a similar condition was found in the Lepidoptera by Mercer (1900).

In the earliest stages of the wing, in *T. plastographus* and *D. valens* and in a Buprestid, even before the fundament becomes recognizable in some specimens, two branches extend from the tracheal trunk to the hypodermis at the place where the wing fundament is shortly to arise. Sections of larvæ taken at this time show the tips of these tracheal branches abutting directly against the bases of the cells of the newly forming wing, the tracheal cells proliferating and spreading out over the inner face of the disc. A mass of cells is thus formed, the walls of which are either very thin or more or less degenerated and in this mass of cytoplasm and nuclei, forming tracheoles are to be seen (Figs. 2, 3, 23). In some cases (*T. plastographus*) the tip of the tracheal branch, when it touches the wing disc, apparently pierces the basement membrane (Fig. 5) and then spreads out directly against the bases of the cells of the disc as a mass of cells, in the cytoplasm of which tracheoles are developed as fine somewhat coiled tubes (Figs. 5, 6). In other specimens the basement membrane apparently degenerates so that the bases of the cells of the disc are free. I have seen this in *D. valens* (Fig. 23). Occasionally the tracheæ push into the disc so that they are nearly surrounded by the cells of the latter (Fig. 1). Fig. 5 (*T. plastographus*) is a section through the disc of the fore wing near its cephalic margin, just after the last moult and shows a tracheal branch pushing through the basement membrane and spreading out under it.

My observations confirm those of Tower and Mercer that the tracheoles are formed from the cytoplasm of thé tracheal cells and not from their nuclei. The manner of development of the tracheoles is the same in all the beetles examined. Figs. 3 and 23 show an early stage in their development. From these figures it will be seen that they are formed as long curved and coiled tubes. As the cytoplasm of the tracheal cells becomes used up in their development, these tubes become looped around the nuclei and by the time that the wing is well evaginated the greater part of the cytoplasm of the cells has been used up and we find a mass of large tracheoles looped and coiled around the nuclei (Fig. 7), nearly every nucleus being closely enfolded by a tracheole.

The tracheoles are present throughout the larval development of the wing and probably degenerate some time during the pupal period. Fig. 17 shows a bundle of tracheoles extending into the base of a wing in the prepupal period. Figs. 13, 15 and 16 show tracheoles that have developed from the wing tracheæ after their entrance into the wing. I have not observed the earliest stages of the formation of these secondary tracheoles, but it will be seen from these figures, which were drawn from the same wing as Fig. 17, that there is no difference in the structure of the primary and the secondary tracheoles. In fact the two cannot be distinguished when in the wing.

The simplest condition of the tracheoles was found by Tower to be in *Coccinella bipunctata*, in which a few tubules developed from the tracheal trunk at irregular intervals, while the most specialized condition was found in the Buprestidæ. My observations confirm those of Tower that the most specialized condition of the tracheoles is to be found in the Buprestidæ, but I find, however, that as in *T. plastographus* and *D. valens*, the tracheoles begin to develop as soon as the wing disc, and are fully formed, as a mass of tubes which are very small next the wing disc, becoming larger further out, by the time that the wing begins to evaginate (Fig. 19).

The tracheoles in the Coleoptera are of a simpler type than is found in the Lepidoptera as described by Gonin (1894), Mercer (1900) and others. Mercer found the tracheoles beginning to develop during the fourth larval stage and becoming functional after the next moult, at the beginning of the last stage. In the Coleoptera, according to Tower, they do not begin to develop until the wing is well formed, during the last larval stage, becoming functional early in the prepupal period and without a moult. I have not been able absolutely to determine the time of their becoming functional in the beetles worked on, but it seems certain that they do so at an early stage in the development of the wing, probably at the moult preceding the evagination of the wing, and their main purpose is to supply the disc with an abundance of air at this important stage of its development.

(*b*) *The Tracheæ of the Wing.* — Shortly before the beginning of the prepupal period, several branches arise from the main trachea and push into the wing, extending nearly to its margin. These are the permanent wing tracheæ, and they are accompanied into the wing by the tracheoles, which begin to uncoil at this time. These tracheæ correspond in number and general position to the veins of the pupal and adult wing, and their early development, taking place soon after the evagination of the wing begins, is similar to what occurs in the wings of Heterometabolous nymphs. According to Comstock and Needham, "in wings developing externally like those of a dragon-fly one sees the principal tracheæ passing very early out into the wing bud, branching freely and forming by multitudinous terminal anastomoses a network of capillary tracheoles. In the beetle wing these fine tracheæ and tracheoles follow rather closely the course of the vein cavities, and are most readily seen by an external examination of the wing just after pupation.

III. THE ORIGIN OF THE WINGS.

The question of the origin of the wings of insects is one over which there has been much controversy. In all insects the wings first become recognizable as slight thickenings of the hypodermis in the pleural region near the place where the suture arises between the dorsum and the pleurum, but whether they have been modified from some other structure or have been developed as entirely new structures is uncertain.

There have been three theories advanced to account for the origin of the wings of insects:

1. That they have been developed from tracheal gills.

2. That they have arisen as lateral outgrowths of the tergum or pleurum of their respective segments.

3. That they arise from degenerated spiracle discs of the meso- and metathorax.

The theory that the wings of insects have arisen from tracheal gills was first advanced by Gegenbauer and adopted by Lubbock and has since been advocated by Pratt (1897). This theory, of course, presupposes that the ancestors of the winged insects were aquatic forms and that still farther back in the line of descent the pregenitors of these aquatic forms must have been terrestrial, in order to have evolved a tracheal system in the body, from which later, tracheal gills could be developed ; as otherwise, if gills had been developed at all by these primitive insects they would certainly have been blood gills.

But all the evidence points strongly to the supposition that the ancestors of the winged insects were terrestrial. It has been shown by Packard (1898) that tracheal gills are adaptive, secondary, temporary larval structures that do not persist in the adult and "are not ancestral, primitive structures." Tower also rightly objects that there is no resemblance between the fundamental type of wing venation, as established by Comstock and Needham, and the tracheation of any known tracheal gill, which should certainly be the case if the wings had been developed from any such structure.

The second theory and the one to which it seems to me that all the present evidence points strongly is the one put forward by Graber (1867) and Muller (1875) and strongly supported by Pancritius (1884) and Packard (1898), that the wings have arisen as simple outgrowths or evaginations of the integument at the suture between the dorsum and the pleurum. After working on. the development of the wings in the Termites, Muller declared that the wings of insects have been derived from lateral continuatuons of the dorsal plates of their respective segments.

I have shown that in the Coleoptera the wing arises on the pleurum, at or near the future position of the dorso-lateral suture, as a thickening of the body wall, which, in the simplest type, begins as a simple pushing outward and downward of this thickening (Fig. 4). In another paper I shall show exactly the same process in the beginning wing of the Neuropteron, *Raphidia* sp. the wing developing as an outpushing of the thickened hypoderm at the lateral fold. In certain Hemiptera, sections of the early stages of which I have examined, it was evident that the wing developed as a simple pushing out and folding of the body wall near the lateral suture.

All this is in line with the conclusion of Packard (1898) that "the wings are essentially simple dorsal outgrowths of the integument, being evaginations of the hypodermis," and of Comstock and Needham (1899) that the "wings arise as sack-like folds of the body wall at the point where the suture between the tergum and the pleurum later develops. In most insects with an incomplete metamorphosis they are so directly continuous with the tergum and become so solidly chitinized with it that they have generally been interpreted as outgrowths from its caudo-lateral margin.'' Muller (1875) has show that in *Calotermes* the wings arise on the meso- and on the metathorax in same position and cannot be distinguished in their early stages from the prominent lateral fold that develops on the prothorax.

In every insect, both in the Holometabola and in the Heterometabola, in which the early stages of the wing development has been investigated, it has been found that the wing arises in a homologous position on the pleurum and that in the simplest types it develops as a simple outpushing of the hypodermis. In fact, in *T. plastographus* and in *D. valens* in their early stages the wing discs are exactly like the leg discs and have a similar mass of tracheoles at their base. While it is not doubted but that the legs have been developed from folds or outpushings of the hypodermis, there is no more reason to believe that the wings have developed from spiracles or in some other circuitous manner than there is in the case of the legs.

The third theory, which was advanced by Verson (1890, 1894), is that the wings develop from the discs of the degenerated spiracles of the meso- and metathorax. This theory has been strongly supported by Tower (1903).

Wheeler (1889), working on a Chrysomelid beetle, *Leptinotarsa 10-lineata*, observed that during the development of the embryo, every segment of the thorax and abdomen develops a spiracular invagination, that these invaginations send off branches some of which unite to form the lateral tracheal trunk, that after the formation of the trunk the prothoracic spiracle closes over and disappears, which is also the case with the metathoracic spiracle, while the mesothoracic spiracle is situated near the suture between the pro- and mesothorax ''and in later stages often has the appearance of belonging to the first segment.'' These observations were confirmed by Tower, who makes the additional statement that in the migration cephalad of the mesothoracic spiracle, ''the spiracle alone migrates and the thickened area of the hypodermis remains and probably becomes the fundament of the elytron.'' He says that after the formation of the longitudinal tracheal trunks ''the openings in the meso- and metathorax are rapidly cut off, leaving a disc-shaped mass of cells which have a somewhat concentric arrangement. The further stages in the degeneration of these spiracles I have not been able to observe. That this rudiment of the spiracle is converted into the imaginal disc of the wing seems probable, however, for the following reasons: (1) The disc of the wing always appears in exactly the same area as that in which the spiracle arose and degenerated, (2) the wing disc frequently shows a concentric arrangement of the cells in early stages but loses this before invagination to form the wing begins, (3) if the wing fundament is not derived from the remains of the spiracle, then, since the wing disc has the exact position occupied by the spiracle, the latter must entirely degenerate and be replaced by new hypodermis, and from this the wing must arise. There is, however, absolutely no ground for belief in such a process and the only conclusion that seems at all tenable is that the wing fundament is derived directly from the remains of the spiracle.'' Tower states farther that these spiracle discs are quite distinct in the embryo shortly before hatching, but that soon after the larva emerges they either degenerate or subside into a quiescent period so that they are not recognizable from the surrounding hypodermis, but that after this period of rest, the cells begin to grow again and form the wing fundament.

I shall present what, I think, is conclusive evidence that the wings do not arise from discs of degenerated spiracles, but which points strongly to the supposition that .the wings have originated as outpushings of the hypodermis at the suture between the pleurum and the tergum.

I have found (1) from the examination of a large number of species of Coleopterous larvæ that the mesothoracic spiracle is present in many species and that in some of these it is functional, while in others the connection with the longitudinal tracheal trunk has more or less completely degenerated but that the spiracle itself is present though considerably smaller than the other spiracles and but lightly chitinized. (2) That in the larvæ of some winged insects possessing both meso- and metathoracic spiracles these spiracles have not migrated forward to any appeciable extent, in some cases occupying exactly the same positions in their respective segments that the abdominal spiracles occupy in their segments. (3) That the wing fundament arises distinctly either above or below the positions occupied by the thoracic and abdominal spiracles. That is to say, the positions of the spiracles on the pleuræ of their respective segments varies in different insects, being in some cases distinctly below the origin of the wing and in others distinctly above the wings, and that they generally lie in the cephalic half of their segments, while the wing fundament is centrally situated on its segment. (4) If it can be proven that in any winged insect the mesothoracic and metathoracic spiracles are present and functional, it seems certain that the wings have originated independently of the spiracles and not from any spiracle disc, since it seems pretty hard to believe, as has been stated by Tower, to be the case with the mesothoracic spiracle, that the spiracles migrate forward while the discs remain behind; that is to say, the chitinized opening alone moves forward and new hypodermal cells become specialized and take on the function of secreting the chitin of the spiracle, while the old cells (disc) remain behind and later develop into a wing.

1. *The Spiracles.*

In the primitive insects, the ancestors of our present winged forms, a pair of spiracles was present in each of the three thoracic segments, as well as in each of the first nine abdominal segments. The presence or absence of certain of these spiracles and their position on the segments has an important bearing on the theory of the origin of the wings from spiracle discs.

While the prothoracic spiracle is present in the embryo, as has been shown by Wheeler, it entirely degenerates and is not known to be present in the larva or adult of any winged insect. In the Lepidoptera however it is said to have been transformed into the spinneret.

The mesothoracic spiracle is always present and open except in some aquatic larvæ. This spiracle has often been called the prothoracic because it has often migrated into that segment, but that it is really the mesothoracic has been proved by Wheeler (1899), whose observations have been confirmed by Tower (1903). This is usually the largest of all the spiracles, but in the aquatic forms, many of the Odonata, Ephemerida and Diptera, this, as well as all the other spiracles of the body are closed or nonfunctional, reappearing as functional spiracles in the adult insect.

In the larvæ of many insects the metathoracic spiracle has entirely degenerated, but this is by no means the rule. Among the Coleoptera it has been proved by Graber (1888) for *Melolontha* and *Lina* and for *Doryphora* by Wheeler (1899) that it is present in the embryo. They found however that this spiracle degenerated before the larva emerged from the egg. Complete degeneration however does not always take place. A careful external examination will show, in the larvæ of many beetles, that the metathoracic spiracle is present, though usually much smaller than the other spiracles and generally much less heavily chitinized, so that it is quite inconspicuous and easily overlooked and it is in many cases evidently closed or non-functional, at least during the larval state. I have found the metathoracic spiracle present in the larvæ of a Trogositid, *Thymalus marginicollis* (*fulgidus*) (Fig. 25), of a Pyrochroid sp., of an Erotylid sp. (Fig. 26), in the larvæ of several unknown Tenebrionidæ, Carabidæ and Cerambycidæ. In the larvæ of the Scolytids *T. plastographus* and *D. valens* (Figs. 32, 33) I have been unable to find the spiracle by external examination, but cross-sections of the metathorax at the place where the spiracle should be show the spiracular branch extending out from the lateral tracheal trunk entirely through the hypodermis (Fig. 12) and they are probably functional. In the larva of *Thymalus marginicollis* the metathoracic spiracle can be plainly seen as a small oval disc with a slit in it and it is probably functional (Fig. 25). Breed (1903) has figured this spiracle in both the larva and the pupa of this beetle. In the larva of the Erotylid sp. (Fig. 26) this spiracle is even larger and though considerably smaller than the other spiracles is undoubtedly functional.

In larvæ that are light colored and not heavily chitinized, such as many of the wood borers, the tracheal system can usually be well brought out by clearing in cedar oil. Fig. 24 shows the lateral tracheal system of a larva thus prepared, of a Ptinid, *Ozognathus cornutus*. It will be seen that under the metathoracic and each of the abdominal spiracles there is a tracheal center, that is, the group of tracheal branches which, in the embryo, arose from the end of each spiracular invagination and ramified in all directions. Although, on account of the small size of the larva, I could not make out with certainty a spiracular opening in the metathorax, there is a tracheal center under the spot where this spiracle should be, which is larger than any in the abdomen except the first and it is not probable that this would be the case if there were no functional opening. It will be seen that, while the mesothoracic spiracle has migrated forward into the suture, the tracheal center migrated with it, while the metathoracic tracheal center is distinctly in the metathorax and occupies nearly the same position in its segment as is occupied by the abdominal centers in their segments.

Among the Hymenoptera I have found the metathoracic spiracle present in the larvæ of the honey bee, *Apis mellifica*, of a wood-boring wasp and of an undetermined mud-wasp. In all cases this spiracle is not to be distinguished from the other spiracles of the body, either in shape, size or position.

I have also found vestiges of the metathoracic spiracle present in the larva of the silk-worm, *Bombyx mori*.

In the Neuroptera I have found the metathoracic spiracle present and open(?), though small, in the larva of *Raphidia* sp., and also in the pupa.

There are numerous references in entomological literature to two pairs of thoracic spiracles. Among the Coleoptera both meso- and metathoracic have been recorded for the larva of *Elmis* (Parnidæ) and *Lycus* (Lampyridæ) (Packard). In the Hymenoptera both pairs are present in the Aculeata and in the Siricidæ, though in the latter the metathoracic is sometimes closed. Both spiracles are also present in *Apis* and *Hylotoma* (Packard). Packard figures the larva of *Bombus* with a full-sized metathoracic spiracle and Bugnion has done the same for *Encyrtus fuscicollis*. Packard also figures a meso- as well as a metathoracic spiracle in a locust, *Melanoplus femur-rubrum*. Calvert (1893) has found that in all the adult Odonata the metathoracic spiracle is present and very distinct. In those insects, the larval stages of which are passed in the water and which develop tracheal gills (Odonata and Ephemerida), thoracic gills are present and open in the early larval stages, but later become closed (Packard). Both pairs of spiracles have been shown by various authors to be present in the Hemiptera. Among the Diptera both pairs are present in the larvæ of Bibionidæ and Cecidomyidæ (Miall and Hammond). Both pairs are present in the Termites.

2. *The Position of the Thoracic Spiracles and their Relation to the Wing Discs.*

During embryonic life the spiracles are formed as invaginations on the pleuræ of their respective segments at a point nearly midway between the middle of the segment and the suture in front of it. This embryonic position of the spiracles can be readily seen by reference to the figures of Graber (1891) who worked on the embryonic stages of the Lepidoptera, Coleoptera and Orthoptera and the figures of Wheeler (1889) for the Coleoptera. The mesothoracic spiracle often migrates forward so that in the larva it lies in the suture or even in the prothorax. This is especially true of the Lepidoptera. The metathoracic spiracle, when present, sometimes migrates forward as far as the suture, while the abdominal spiracles as a rule, remain very nearly in their embryonic positions.

In the Coleoptera the mesothoracic spiracle usually lies in or near the suture between the pro- and mesothorax (Figs. 24, 25), but in many cases it is distinctly in the mesothorax and in some species there is little or no migration (Fig. 26). Its position corresponds very nearly to that of the mesothoracic (Figs. 25, 26), but I have never found it entirely in the mesothorax.

The height at which the spiracles are situated on the pleurum is a little below the place where, later, the suture is formed between the dorsum and the pleurum and is always distinctly below the wing discs (Fig. 31). The latter arise near the future sutural line and are always centrally situated on their segments, while the spiracles lie in the front part. Their relative positions can be determined by certain muscles occurring in each segment. In certain Hymenoptera, however, the spiracles are situated at some distance above the wing discs.

Vestiges of the metathoracic spiracle are to be found in the larvæ of some Lepidoptera. An external examination of the silk-worm (*B. mori*) will reveal the remains of this spiracle as a small, oval, faintly-chitinized ring, occupying a position on the pleurum of the metathorax near the suture between that segment and the mesothorax and in a position very nearly corresponding to that of the first abdominal spiracle on its segment, as will be seen by reference to Fig. 27 (drawn from a larva killed after the second moult and cleared in cedar oil). This shows the lateral tracheal system of the thoracic and first abdominal segment and the forming wing buds. It will be seen that the latter are centrally situated on the sides of their respective segments and dorsad of the longitudinal tracheal trunk. A small tracheal branch arising near the first abdominal spiracle pushes forward to the metathoracic wing disc, where it is met and joined by another small branch arising near the metathoracic spiracle. Two tracheæ arising similarly, near the meso- and metathoracic spiracles respectively, join at the center of the mesothoracic wing disc (Fig. 27). These two tracheal branches probably give rise, the one to the radio-medial and the other to the costo-anal groups of wing tracheæ. It is evident from the position of these wing discs that they have not arisen from any part of the spiracles. The metathoracic wing disc is not situated over the tracheal center of its segment, while the remains of the metathoracic spiracle does lie over this center. The mesothoracic spiracle has migrated forward into the prothorax, but it is evident that not only the opening but the spiracular disc, as well as the tracheal center have migrated forward. Moreover, the metathoracic spiracle has not migrated, or at least very slightly, as will be seen by comparing its position with that of the first abdominal spiracle ; so that it is not possible that either the meso- or metathoracic wing should have arisen from any part of a spiracle.

While, among many of the orders of insects, the metathoracic spiracle is in a more or less vestigial condition, being either considerable smaller, or entirely degenerated, it is to be found in the Hymenoptera, full sized and perfectly formed. In this order the positions of the wings and the spiracles in respect to one another are different from anything that I have found in any of the other orders. In the larva of the honey-bee (*Apis mellifica*) the metathoracic spiracle is as large as any of the other spiracles and cannot be distinguished from them in shape. The meso- and metathoracic spiracles and the abdominal as well occupy positions on the pleurum near the front margin of their respective segments, while the wings are centrally situated on their segments and arise considerably below the spiracles, as may be seen by reference to Figs. 28, 29. This is just the reverse of their position in the Coleoptera and Lepidoptera, in which, I have shown (Fig. 27), the wing discs are distinctly above a line drawn through the spiracles, while in *Apis*, the base of the wing bud is below the spiracles, at about one third the distance between them and the leg buds (Figs. 28, 29). This can be easily seen by an external examination of the larva, as the wing and leg buds lie outside the hypodermis, next the cuticle and can be readily seen through it. The tracheal centers under each spiracle are greatly reduced, there being only a few small branches which soon break up into a fine network of small tracheæ or tracheoles. We find in *Apis*, just as we did in the Silkworm, a small trachea running from the tracheal center behind each wing bud and meeting at the base of the bud a similar trachea coming from the tracheal center in front of the wing. I believe this will be found true of all winged insects, while from these two tracheæ evidently develop, respectively, the two groups of wing tracheæ.

V. CONCLUSION.

It has been shown in this paper that among the Coleoptera are to be found examples of the simplest type of wing development known to occur in the Holometabola, while this type differs from the development of the wings in the Heterometabola mainly in that it is held closely to the side of the body until pupation, and that in the Scolytidæ and in certain Bruchidæ and Buprestidæ, the development of the wing takes place without any preliminary invagination of the center of the disc or recession from the cuticle. It has been shown, also, that there is a distinct system of tracheoles developed at the base of the wing, the first appearance of which is coincident with the forming of the wing disc, and that these tracheoles cannot be distinguished from those that arise within the wing from the permanent tracheæ, during the prepupal period.

I have shown conclusively that the wings do not arise from any part of the spiracles of the mesothorax or metathorax, nor do the spiracles and the wings arise from the same place on the pleurum, even in those insects in which the metathoracic spiracle degenerates. But that the spiracles arise in the embryo in a different position from that in which the wings arise, while in many insects the metathoracic spiracle does not degenerate.

In those insects possessing tracheal gills, these gills are developed in larval or pupal life and are temporary, adaptive structures that do not persist in the adult, while there is no evidence whatever to show that the wings have been derived from any such structure possessed by the ancestors of the winged insects.

On the other hand, the only conclusion that seems at all reasonable, and the one to which the earliest stages in the formation of the wing in all insects seems to point, is, that the wings have been derived as lateral outgrowths or folds of the hypodermis of the pleurum or tergum, or both.

METHODS.

In preparing the material for these investigations several different methods were tried. Among the fixing solutions, the best results were obtained with a saturated solution of corrosive sublimate in ten per cent. formalin, washed out with four per cent. formalin. Very good results were also obtained by the use of Tower's No. 3, the formula for which is as follows:

Sat. sol. $HgCl_2$ in 35 per cent. alc. .. ..60 vols.
Glacial acetic acid (99.5 per cent.)........................10 "
Platinic chloride 2 per cent. sol. in aq. dest............30 "

This can be used either warm or cold, while the material should be cut into as small pieces as possible.

Out of a number of different stains tried, I selected Ehrlich's acid hæmatoxylin as the best all round stain for the wings and other hypodermal tissues, though Delafield's hæmatoxylin gave good results and iron hæmatoxylin worked well in some cases.

EXPLANATION OF PLATES XI–XVII, VOL. XII.

All the figures used in this paper were outlined with a camera lucida and the details put in with free hand. The figures are arranged dorsal side up, and the anterior surface toward the reader.

Abbreviations used in the figures:

b, basement membrane.
c, head.
ct_1, old cuticle.
ct_2, new cuticle.
ct_3, secondary cuticle.
f.b, fat body.
h, hypodermis.
h.sp, spur of hypodermis.
l.b, leg bud.
le'cy, leucocyte.
l.tr, lateral tracheal trunk.
ms, muscle.
nc, nucleus.
s_1, mesothoracic spiracle.
s_2, metathoracic spiracle.
s_3, first abdominal spiracle.
s_8, last abdominal spiracle.
set, seta.
tr, trachea.
tr.cl, tracheal cells.
tr'ol, tracheole.
tr'ol.w, wing tracheole.
tr.cr, tracheal center.
w.d, wing disc.
w.b, wing bud.
w.l, wing lumen.

Fig. 1. *Tomicus plastographus.* Just starting wing disc, showing tracheæ partly surrounded by cells of the disc. From cross-section of larva in the middle of the second stage.

Fig. 2. *T. plastographus.* Not quite frontal sagittal section of wing disc, showing forming tracheoles at base of disc. From larva in middle of second stage.

Fig. 3. *T. plastographus.* Wing disc, showing the pronounced folding and ridging of the cells. From cross section of larva just before second moult.

Fig. 4. *T. plastographus.* Wing disc just beginning to evaginate. From cross section of larva soon after second moult.

Fig. 5. *T. plastographus.* Cross-section of wing near cephalic end, showing trachea pushing through basement membrane, the cells spreading out underneath it and tracheoles beginning to form. From larva just after second moult.

Fig. 6. *T. plastographus.* Cross-section through middle of same wing from which Fig. 5 was taken, showing forming tracheoles and the bases of the cells beginning to elongate and separate.

Fig. 7. *T. plastographus.* Cross-section of wing well evaginated, showing thread-like bases of cells and tracheoles well developed.

Fig. 8. *T. plastographus.* Cross-section of wing through a vein cavity, showing wing extending to base of leg bud.

Fig. 9. *T. plastographus.* Cross-section of wing, showing beginning of folding of wing and the pulling of the nuclei into one row. Tracheæ and tracheoles in the vein cavities.

Fig. 10. *T. plastographus.* Cross-section of part of wing from same larva as Fig. 9, showing elongated bases of cells and tracheæ and tracheoles in vein cavity.

Fig. 11. *T. plastographus.* Full grown wing, shortly before pupation.

Fig. 12. *T. plastographus.* Cross-section through the metathoracic spiracle. (The cuticle was pulled away from the hypodermis during sectioning.)

Fig. 13. *T. plastographus.* Tracheole from full grown wing.

Fig. 14. *T. plastographus.* Tracheoles formed around the nuclei, from base of wing just before second moult.

Figs. 15 and 16. *T. plastographus.* Tracheoles from prepupal wing.

Fig. 17. *T. plastographus.* Bundle of tracheoles entering wing. From larva in prepupal period.

Fig. 18. Just starting wing disc of a Buprestid.

Fig. 19. Wing disc just beginning to evaginate, showing great proliferation of tracheoles. From same Buprestid as Fig. 18.

Fig. 20. *Bruchus* sp. Cross-section of wing well evaginated, showing trachea in vein cavity and the thick walls of the wing bud.

Fig. 21. *Bruchus* sp. Wing at a later stage than Fig. 20. The basement membrane has nearly degenerated.

Fig. 22. *Dendroctonus valens.* Full-grown wing shortly before pupation.

Fig. 23. *D. valens.* Forming wing disc, just before last moult, showing folding of the disc and the tracheoles at base.

Fig. 24. *Ozognathus cornutus.* Lateral tracheal system from a larva cleared in cedar oil.

Fig. 25. *Thymalus marginicollis* Chev. Lateral view of front part of body of a full-grown larva, showing metathoracic spiracle.

Fig. 26. Lateral view of meso- and metathorax of a larva of an Erotylid, showing meso- and metathoracic spiracles and their positions.

Fig. 27. *Bombyx mori.* Lateral view of larva just after second moult, cleared in cedar oil, showing lateral tracheal system of thorax, the vestiges of the metathoracic spiracle and the wing discs.

Fig. 28. *Apis mellifica.* Lateral view of head, thorax and first abdominal segment, showing the leg and wing buds as seen through the cuticle, and their relation to the spiracles. From larva one half grown, cleared in cedar oil.

Fig. 29. *Apis mellifica.* Larva (prepupal period early) showing relative positions of spiracles and wing buds, as seen through the cuticle.

Fig. 30. From same larva as Fig. 29. Ventral view showing wing and leg buds.

Fig. 31. *Tomicus plastographus.* A not quite cross-section of a larva just before last moult, showing hinder margin of metathoracic wing disc and part of first abdominal spiracular trunk, to illustrate the relative heights on the pleurum of the spiracles and wing discs.

Fig. 32. *T. plastographus.* Full grown larva; side view.

Fig. 33. *Dendroctonus valens.* Larva; side view.

Fig. 36. *Dendroctonus valens.* Cross-section of wing disc, showing an early stage of the evagination.

Figs. 34, 35 and 37. *Tomicus plastographus.* Early stages in the evagination of the wing.

Fig. 38. *T. plastographus.* Cross-section of wing bud showing thread-like bases of the cells and a trachea pushing into the wing. From larva at about the beginning of the prepupal period.

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Complete bibliographies of the literature on wing development can be found in Packard's Text Book and Mercer's (1900) and Tower's (1903) papers.

Class I, HEXAPODA.

Order IV, DIPTERA.

A SYNOPTIC TABLE OF NORTH AMERICAN MOSQUITO LARVÆ.

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I have had this table in hand for over a year, but have been dissuaded from printing it by Dr. Howard, who advised delay on the ground that new forms were continually being found. Now, however, we have at hand all the larvæ of the known species of the Atlantic Coast region with the exception only of a few rare or doubtful forms, namely *Culex niveitarsis* Coq. and *C. onondagensis* Felt, recently described, *Anopheles nigripes* Staeg., *A. bifurcatus* Linn. and *Culex squamiger* Coq., of doubtful or recently recorded occurrence and *Culex hirsuteron* Theob. and *C. testaceus* Wulp., of doubtful identity.

There are a number of Western species still unknown in the larva, while the West Indies and Mexico as well as the Arctic regions are largely unexplored. Still, as the table seems likely to be useful in its present form for the Eastern United States, it is herewith presented.